

Gender and cytoarchitecture differences: Functional connectivity of the hippocampal sub-regions

Adnan A.S. Alahmadi^{a,b,*}, Nada O. Alotaibi^{a,1}, Norah Y. Hakami^{a,1},
Raghad S. Almutairi^{a,1}, Afnan M.F. Darwesh^a, Rawan Abdeen^a, Jamaan Alghamdi^a,
Osamah M. Abdulaal^c, Walaa Alsharif^c, Salahaden R. Sultan^a,
Ibrahim H. Kanbayti^a

^a Radiologic Sciences, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia

^b NMR Research Unit, Department of Neuroinflammation, Queen Square MS Centre, UCL Queen Square Institute of Neurology, London, United Kingdom

^c Diagnostic Radiology Technology, College of Applied Medical Sciences, Taibah University, Madina, Saudi Arabia

ARTICLE INFO

Keywords:

Functional magnetic resonance imaging (fMRI)
Resting state functional magnetic resonance
imaging (rsfMRI)
Functional connectivity
Hippocampus
Gender differences
Hippocampal sub-fields

ABSTRACT

Introduction: The hippocampus plays a significant role in learning, memory encoding, and spatial navigation. Typically, the hippocampus is investigated as a whole region of interest. However, recent work has developed fully detailed atlases based on cytoarchitecture properties of brain regions, and the hippocampus has been sub-divided into seven sub-areas that have structural differences in terms of distinct numbers of cells, neurons, and other structural and chemical properties. Moreover, gender differences are of increasing concern in neuroscience research. Several neuroscience studies have found structural and functional variations between the brain regions of females and males, and the hippocampus is one of these regions.

Aim: The aim of this study to explore whether the cytoarchitecturally distinct sub-regions of the hippocampus have varying patterns of functional connectivity with different networks of the brain and how these functional connections differ in terms of gender differences.

Method: This study investigated 200 healthy participants using seed-based resting-state functional magnetic resonance imaging (rsfMRI). The primary aim of this study was to explore the resting connectivity and gender distinctions associated with specific sub-regions of the hippocampus and their relationship with major functional brain networks.

Results: The findings revealed that the majority of the seven hippocampal sub-regions displayed functional connections with key brain networks, and distinct patterns of functional connectivity were observed between the hippocampal sub-regions and various functional networks within the brain. Notably, the default and visual networks exhibited the most consistent functional connections. Additionally, gender-based analysis highlighted evident functional resemblances and disparities, particularly concerning the anterior section of the hippocampus.

Conclusion: This study highlighted the functional connectivity patterns and involvement of the hippocampal sub-regions in major brain functional networks, indicating that the hippocampus should be investigated as a region of multiple distinct functions and should always be examined as

* Corresponding author. Radiologic Sciences, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah 21589, Saudi Arabia.
E-mail address: aaalahmadi@kau.edu.sa (A.A.S. Alahmadi).

¹ Contributed Equally.

sub-regions of interest. The results also revealed clear gender differences in functional connectivity.

1. Introduction

The hippocampus is among the most vital and intricate brain areas, participating in numerous brain functions such as learning, memory encoding, memory consolidation, and spatial navigation. It is also associated with various neurological disorders, with several neuropsychiatric diseases including Alzheimer's disease (AD), epilepsy, depression, and dementia affecting the hippocampus as the primary and most impacted structure in the brain [1–5]. Functional MRI (fMRI) is an advanced MRI technique that enables the examination of the function of brain regions, including the hippocampus, while an individual performs a task or is at rest. The fMRI signal relies on blood oxygen level-dependent contrast (BOLD) [6,7].

fMRI can provide a great understanding of segregation and integration of brain regions caused by sensory stimulus, cognitive and other task related functions [7,8]. As a result, it allows the study of how the healthy brain functions, how various disorders impact it, how it strives to recover after damage, and how medications can control activity or post-damage recovery [9–11]. fMRI has become the most widely used technology in neuroscience [12]. For example, resting-state functional magnetic resonance (rsfMRI) can be used as a useful tool for the study of abnormal patterns of brain connectivity at rest in patients with Parkinson's disease, multiple sclerosis, and obsessive-compulsive disorder [13–15]. Other examples of using fMRI include understanding the physiology and functions of the human brain in healthy subjects [16].

Using rsfMRI, it has been reported that the hippocampus is involved in a resting state network, known as the default mode network [17]. However, it remains unclear how the hippocampus is functionally connected to different rsfMRI networks and whether its sub-regions have non significant connection or similar connections, as this has not been fully investigated. For example, in a recent study, a more advanced analysis was carried out to investigate five sub-regions of the hippocampus: CA1, CA3, CA4, Dentate Gyrus (DG), and the subiculum (SUB) [18]. It was found that these five sub-regions primarily connect to two different resting-state networks. The somatomotor network is related more to the CA1, CA3, and CA4/DG sub-fields and less to the SUB. On the other hand, the default mode network is more strongly associated with the SUB and CA4/DG sub-fields and less to the CA1 and CA3. In recent years, research has shown that different hippocampal sub-fields exhibit varying degrees of functional connectivity (FC) to specific brain networks, such as the default mode network (DMN), frontoparietal network (FPN), visual network, and sensorimotor network [19–21]. For instance, FPN demonstrates a smaller spatial extent of significant FC with hippocampal sub-fields compared to the visual network, sensorimotor network, and DMN [20]. Additionally, certain hippocampal sub-regions show stronger functional connections to the DMN, while others exhibit stronger connectivity to the visual network [20].

Furthermore, neuroscientists are increasingly concerned with gender differences in various aspects [22,23]. Several neuroscience studies have found structural and functional differences between the brains of females and males, which may contribute to gender disparities in behavior and cognition [24,25]. In terms of gender differences in the anatomical structure of the hippocampus, it has been confirmed that males have a larger presubiculum, fimbria, hippocampal fissure, and parasubiculum, while females have a larger hippocampal tail [26]. This study also showed that there were no gender differences in the CA2/3, CA4, HATA, or GCDG sub-fields. Additionally, some studies have identified that females had more extensive functional connectivity than males, especially in the prefrontal cortex, hippocampus, and amygdala when involved in emotional processing [27,28]. Other studies have reported lower functional connectivity and higher node efficiency in the hippocampus and parahippocampus of males [29]. It has been indicated that females have higher functional connectivity than males in the ventral precuneus and other regions of the default mode network [30].

As mentioned in the previous studies, the hippocampus is an area of interest for investigating its connections to different brain networks, as well as gender differences. Recently, a new study has developed fully detailed anatomical atlases based on the cytoarchitecture properties of brain regions, and sub-divided the hippocampus into sub-areas that vary in terms of the number of cells, neurons, and other chemical properties [31–33]. The sub-divided hippocampal regions include seven distinct structural sub-regions, consisting of four cornu ammonis regions (CA1–4), the dentate gyrus (DG), the subiculum (SUB), the entorhinal cortex (EC), and the hippocampal-amygdaloid transition (HATA).

Based on these factors, the sub-divisions of the hippocampus based on cytoarchitecture properties as well as the gender differences in terms of functional connectivity, this study aims to explore the functional connectivity of 7 hippocampal sub-regions using a priori defined cytoarchitecture atlas [31–33] with major brain rsfMRI networks and investigate how these functional connections differ based on gender differences. We hypothesize that the degree of functional connectivity between the 7 hippocampal sub-regions and the 8 major brain networks will differ depending on the sub-region, and that sub-region-specific differences in connectivity will differ by gender, such that females exhibit stronger functional connectivity between certain sub-regions and brain networks compared to males.

2. Materials and methods

2.1. Participants

The study included 200 healthy volunteer subjects, 100 male and 100 female, who were age-matched university students and right-handed, with no neurological or psychiatric disorders. The mean ($\pm$ SD) age was 20.9 ± 1.5 (range: 18–24) years for males and 20.8 ± 1.3 (range: 18–24) years for females. The data used in the study was obtained from an open-access dataset collected and downloaded

from the NeuroImaging Tools & Resources Collaboratory, based on the Wakayama data study [34]. For more information, please visit the website at https://www.nitrc.org/projects/wakayama_psyrs.

2.2. MRI data acquisition

All participants underwent MRI acquisition on a weekday afternoon from 1 p.m. to 5 p.m. A 3 T MRI scanner (PHILIPS, the Netherlands) with a 32-channel head coil (SENSE-Head-32CH) was used to scan the subjects. T1-weighted images were obtained using the following parameters: TR = 6.9 ms, TE = 3.3 ms, FOV = 256 mm, matrix scan = 256, slice thickness = 1.0 mm, and flip angle = 10°. Functional images were obtained using the following parameters: TR = 3000 ms, TE = 30 ms, FOV = 192 mm, matrix scan = 64, slice thickness = 3.0 mm, and flip angle = 80°, resulting in 107 vol

2.3. MRI data preprocessing

The preprocessing of rsfMRI data was achieved using Statistical Parametric Map (SPM12) and CONN [33,35]. The preprocessing steps aimed to remove artifacts and ensure that the model assumptions were accurately tested. Volume-based preprocessing steps for rsfMRI included realignment, slice-timing correction for inter-slice differences in acquisition time, co-registration between the functional volumes and T1 map for each subject, normalization of the functional volumes to the MNI template using the T1 co-registered images, and smoothing the functional volumes with an 8 mm³ smoothing kernel. For motion correction, we implemented a two-pass realignment procedure in SPM12. In the first pass, all functional volumes were realigned to the mean functional image. In the second pass, the functional volumes were realigned to the first volume, which was subsequently used as a reference for co-registration with the T1-weighted image. We calculated frame-wise displacement to identify volumes with excessive motion. These volumes were flagged for scrubbing in the subsequent denoising step.

To register the functional images to the T1 images, we used a rigid-body transformation in SPM12, which estimated and applied a 6-parameter (3 translations and 3 rotations) spatial transformation. The co-registration was visually inspected for each subject to ensure accurate alignment between functional and anatomical images. If errors were identified, the registration process was repeated with manual initialization. With regards to the motion cutoffs, the default setting of 0.9 mm was used in the motion correction process with the CONN toolbox. Furthermore, Artifact Detection Tools (ART) were utilized to detect and eliminate any instances of motion or other artifacts that could have influenced the data, which involved removing the initial few functional volumes as well. To control for motion

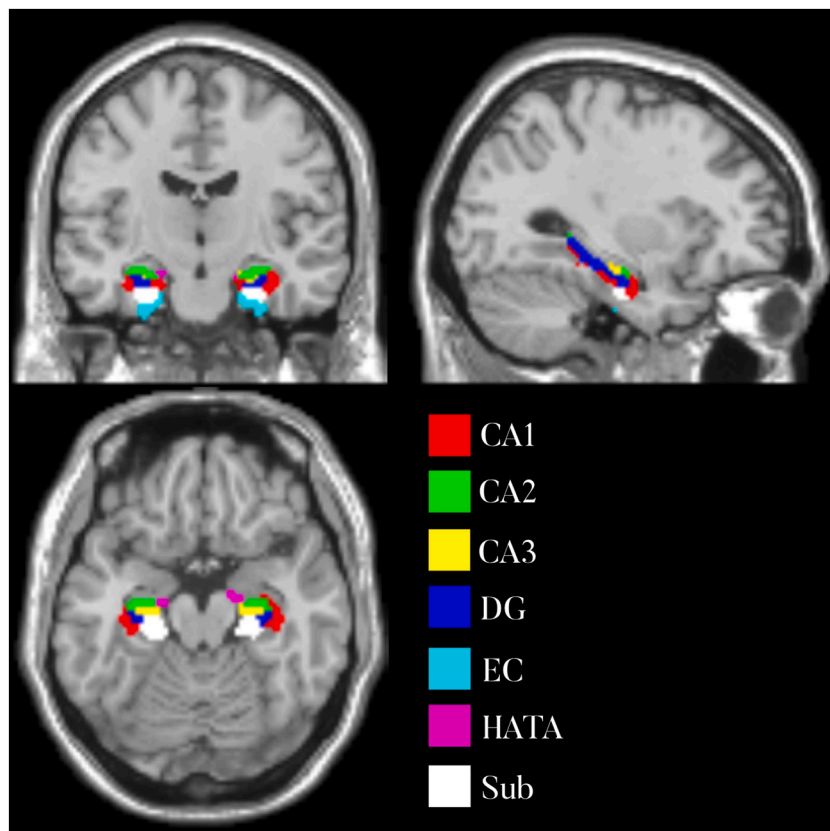


Fig. 1. The 7 hippocampal cytoarchitectonic sub-regions on both hemispheres.

in the group analyses, motion regressors, as well as all the confounders such as white matter and cerebrospinal fluid (CSF), were included in the General Linear Model (GLM) model. This is a standard procedure recommended in the CONN toolbox for controlling for motion and other sources of variance. Moreover, bandpass filtering with a default range of 0.008–0.09 Hz was applied to remove low-frequency drifts and high-frequency noise from the data. The selection of this range was based on our study design and specific requirements. Temporal processing was performed alongside data denoising to remove artefactual confounding effects from the BOLD signal. The movement parameters and other confounding signals, including the flagged volumes for scrubbing, were added as regressors of no interest during the statistical analysis.

2.4. Selection of the regions of interest

The hippocampal seeds included seven sub-regions on both cerebral hemispheres: CA1, CA2, CA3, dentate gyrus (DG), entorhinal cortex (EC), hippocampus-amygdala-transition-area (HATA), and subiculum (sub) Fig. 1. These regions were detected using subcortical atlases in an MNI space [31–33], based on the cytoarchitectonic probability anatomy map from ten post-mortem brains [31–33]. Furthermore, the specific brain areas of interest comprised significant aggregations of functional networks, delineated in CONN. These networks encompassed the default mode, sensorimotor, visual, salience, dorsal attention, language, frontoparietal, and cerebellar networks.

2.5. Statistical analysis

The statistical analysis was conducted in two stages. Firstly, at the subject level, connectivity matrices were computed for each participant, focusing on regions of interest. This involved employing weighted general linear bivariate correlation models to calculate the Fisher-transformed bivariate correlation coefficients between pairs of timeseries from the different regions. At the second level, connectivity matrices were generated through group-level statistical analysis using ROI-ROI (region of interest to region of interest) approach, with appropriate between- and within-group T-tests and/or F-tests, to compare and identify the targeted networks connected to each of the hippocampal sub-regions. The gender of each subject was included as a covariate of interest in the second level analysis. A corrected false discovery rate (FDR) ($p < 0.05$) (multivariate statistics parametric (MVPA) omnibus test) was used as a standardized protocol for the results. By taking into account groups or networks of connected ROIs, functional network connectivity multivariate parametric statistical inferences were utilized to generate cluster-level conclusions. The study is subsequently carried out by examining the comprehensive range of functional connections between the ROIs, encompassing both intra- and inter-network connectivity.

3. Results

This study explores the functional connectivity between the sub-regions of the hippocampus in both hemispheres with different targeted brain networks. The results show that there are positive, negative, or missing connections between each of the hippocampal sub-regions and the brain networks. Additionally, the study investigated how these functional connections of those sub-regions differ in terms of gender differences. Fig. 2(a and b) illustrates the connectivity matrix of the sub-regions of the hippocampal for both left and right hemispheres, as well as the selected functional brain networks for all subjects, regardless of gender. Fig. 3(a and b) and Fig. 4(a and b) show the connectivity matrix of the sub-regions of the hippocampal for both left and right hemispheres, as well as the selected functional brain networks for male and female groups, respectively. Detailed tables of each-sub-region with the specific t or p values as well as the strength of the functional connectivity (effect sizes – beta) with several brain regions or networks are provided in the supplementary materials of this paper.

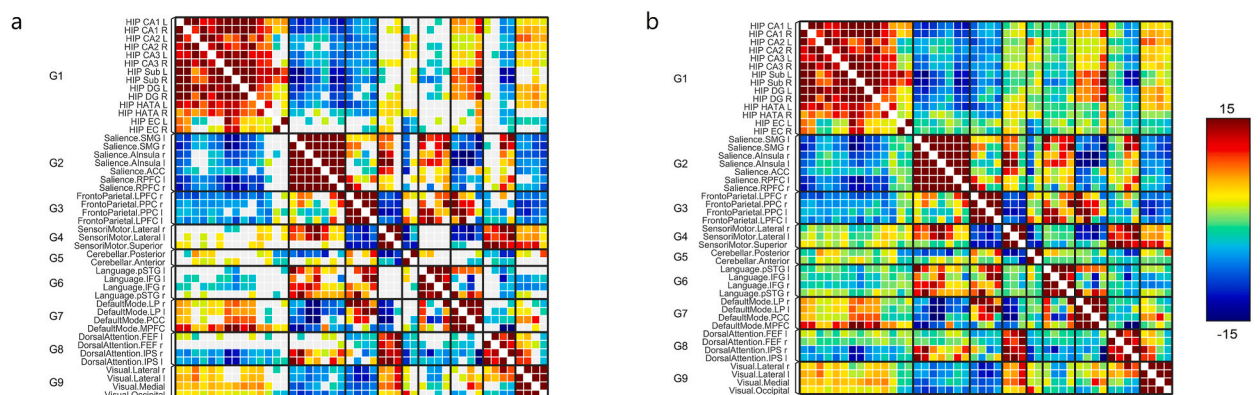


Fig. 2. (a, b) The connectivity matrix of the hippocampus sub-areas as well as the selected functional brain networks for all subjects, regardless of gender differences, is shown. The result is showing the raw findings with thresholding using $p < 0.05$ (a) and without thresholding (b) for the purpose of illustration.

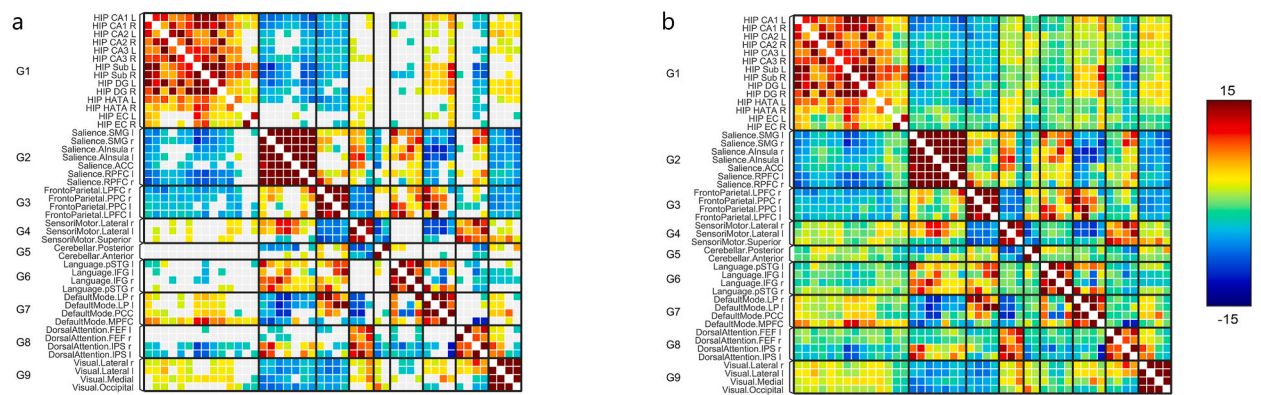


Fig. 3. (a, b) The connectivity matrix of the hippocampus areas as well as the selected functional brain networks for the male subjects is shown. The result is showing the raw findings with thresholding using $p < 0.05$ (a) and without thresholding (b) for the purpose of illustration.

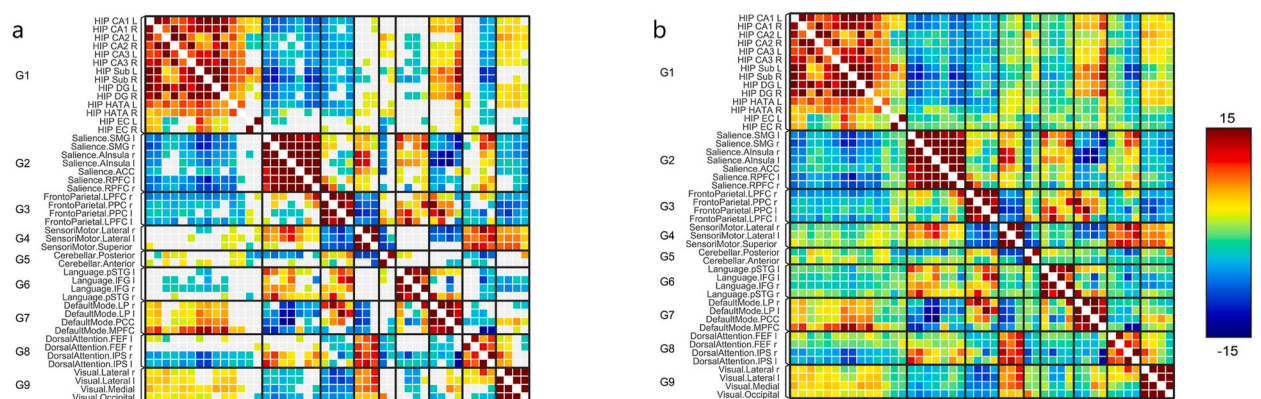


Fig. 4. (a, b) The connectivity matrix of the hippocampus areas as well as the selected functional brain networks for the female subjects is shown. The result is showing the raw findings with thresholding using $p < 0.05$ (a) and without thresholding (b) for the purpose of illustration.

3.1. Overall similarities across the sub-regions of the hippocampus

Upon examining the findings for all subjects, focusing on similar functional connections, all sub-areas of the hippocampus in the left and right hemispheres exhibit positive functional links with the default mode network, especially the medial prefrontal cortex (MPFC). Positive functional connections were also found between the majority of the sub-regions and the regions comprising the visual network, predominantly the occipital and medial networks. The study revealed the presence of negative functional connections between the majority of the sub-regions and the language network regions. Additionally, negative functional connections were observed with the regions associated with the dorsal attention network, particularly the intraparietal sulcus (IPS). The results further demonstrated that most of the sub-regions displayed negative functional connections with the regions related to the frontoparietal and salience networks.

3.2. Overall differences across the sub-regions of the hippocampus

When analyzing the results for all participants and exploring various functional connections, noticeable differences arise in the interactions between different sub-regions of the hippocampus and the brain networks. Most of the sub-regions show either positive or negative functional connections with the components of the language network, but HATA stands out as the only sub-region without any connections to it. Additionally, variations exist between the sub-regions of the hippocampus and the regions associated with the cerebellar network. Negative functional connections with the posterior cerebellum are exclusively found with the right CA1 and EC in both hemispheres. The anterior cerebellum also shows negative functional connections with EC in both hemispheres. Positive functional connections with the anterior cerebellum are solely found with the CA2, left CA3, right HATA, and SUB sub-regions. Furthermore, positive functional connections with the posterior cerebellum are exclusively found in the CA2, left CA3, and HATA sub-regions. Left CA1, right CA3, and DG are the only sub-regions lacking functional connections with the cerebellar network. The study revealed that EC in both hemispheres is the sole sub-region exhibiting positive functional connections with the frontoparietal network. Additionally, right EC is the only sub-region lacking connections with all regions comprising the salience network, while

positive functional connections are exclusively present in left EC and right HATA. CA1, right CA3, and right DG are the only sub-regions demonstrating positive functional connections with the left frontal eye fields (FEF) of the dorsal attention network. The results also indicate that most sub-regions of the hippocampus exhibit positive connections with the regions comprising the sensorimotor network, and the only sub-regions lacking connections with it are the left CA2 and left CA3. Distinctions in functional connectivity to the visual networks are also evident. Among the majority of sub-regions, there is a prevalence of positive connections with the areas constituting the visual network. However, EC stands apart as the sole sub-region that shows a negative connection with the visual network regions.

3.2.1. Similarities among the sub-regions of the hippocampus as a factor of gender

After analyzing the findings to explore functional connectivity similarities between male and female subjects while accounting for gender as a covariate of interest, it becomes evident that CA1, CA2, CA3, DG, and SUB in both hemispheres display positive functional connections with the regions of the default mode network, particularly the medial prefrontal cortex (MPFC). No functional connections are observed between the left DG, right HATA, left EC, and the regions comprising the language network. The results also demonstrate that the posterior cerebellar exhibits a positive functional connection with left CA2, right CA2, and left CA3. No functional connections are found between the regions comprising the cerebellar network and right CA3, as well as right DG. CA1, CA2, CA3, DG, HATA, and SUB in both hemispheres reveal negative functional connections with most of the regions comprising the frontoparietal network. Most sub-regions in both hemispheres demonstrate negative or no functional connection with the regions comprising the salience and dorsal attention network. CA1 in both hemispheres, right EC, and left HATA exhibit a positive functional connection with some regions comprising the sensorimotor network, while left CA2 and left DG lack any connections. Right CA1, left CA2, left CA3, left HATA, and DG in both hemispheres possess positive functional connections with all regions comprising the visual network.

3.2.2. Differences among the sub-regions of the hippocampus as a factor of gender

Upon examining the outcomes to investigate functional connectivity differences among male and female subjects, considering gender as a covariate of interest, the left and right EC sub-regions in males demonstrate positive functional connections with the medial prefrontal cortex (MPFC) of the default mode network. In contrast, in females, EC in both hemispheres lack functional connections with all regions comprising the default mode network. Furthermore, right CA2 and CA3 in both hemispheres of female subjects exhibit positive functional connections with the posterior cingulate cortex (PCC), while in male subjects, those sub-regions lack any connection with the PCC. Left CA2 in male subjects is the only sub-region exhibiting a positive functional connection with the inferior posterior superior temporal gyrus (pSTG I) of the language network. Differences are also evident between the sub-regions and the regions comprising the cerebellar network. For instance, the anterior cerebellum demonstrates positive connections with left CA1, left CA2, left CA3, HATA, and SUB in both hemispheres of female subjects. In contrast, in the male group, positive functional connections are solely observed with the right CA2 and left DG. The posterior cerebellum also demonstrates positive functional connections with HATA sub-regions of the female group in both hemispheres, while in the male group, positive functional connections are present between the posterior cerebellum and the left DG. The sole sub-region exhibiting a positive functional connection with the frontoparietal network is EC in the female group. The results also reveal that left EC sub-regions of males possess positive functional connections with the anterior cingulate cortex (ACC) of the salience network, while the left EC in females lack any functional connections with the ACC. Left SUB, left HATA, right DG, and CA1 in both hemispheres of female subjects exhibit positive functional connections with the left frontal eye fields (FEF) of the dorsal attention network. In contrast, in males, the only sub-region possessing a positive connection with FEF is the right CA1. The outcomes also demonstrate that right CA2 in females lacks functional connections with the regions comprising the sensorimotor network, while in males, the right CA2 has a positive functional connection with the sensorimotor network.

4. Discussion

In this study, a substantial number of subjects was utilized to examine the functional connectivity of the sub-regions of the hippocampus with the major brain networks and to explore how this functional connectivity differs by gender, using rsfMRI. Specifically, 7 cytoarchitectonic sub-regions, including CA1, CA2, CA3, DG, EC, HATA, and SUB, were used as seeds of interest to investigate their functional connectivity to well-defined functional brain networks, such as the default mode, visual, sensorimotor, salience, dorsal attention, frontoparietal, cerebellar, and language networks.

4.1. Similarities and differences among the sub-regions of the hippocampus

The results showed that most hippocampus sub-regions were functionally connected to major brain networks, particularly the default and visual networks. These findings confirm prior studies reporting the significant functional role of the hippocampus in various visual and cognitive functions [36–41]. Given the heterogeneous nature of the hippocampus, which can be divided into several sub-domains along the longitudinal axis with distinct connectivity profiles [42], it is plausible that seeds placed on default mode network regions have specific connectivity patterns with certain hippocampal sub-fields. However, our study demonstrated that all hippocampal sub-regions, not just distinct sub-fields, were positively functional connected with regions of the default mode network. Although some previous functional connectivity studies reported distinct functional connectivity patterns of specific hippocampal sub-fields with default mode network regions, their findings were inconsistent [17,42–44]. The discordant findings might be attributed to the variable neuroimaging techniques and different ways of classifying hippocampus parts used among these studies. For example,

some studies employed seed-based or independent component analysis for functional connectivity analysis, while others used different parcellation schemes for dividing the hippocampus, such as those based on anatomical landmarks, functional connectivity patterns, or cytoarchitectonic properties. These methodological differences can lead to variations in the observed connectivity patterns between hippocampal sub-regions and default mode network regions. In our study, the hippocampus sub-regions were classified based on their distinct cytoarchitectonic and chemical characteristics, suggesting that despite the presence of heterogeneous structural changes, the functional connectivity to the default mode network is strongly connected to all of these sub-regions.

Although previous studies on functional connectivity have demonstrated the hippocampus's direct involvement in visual activities and sensorimotor tasks [36,45,46], none of these studies examined the connectivity of the hippocampus as sub-fields with these networks. The present study shows the precise hippocampal sub-regions that participate in connectivity with the regions of visual as well as sensorimotor networks. In line with the study by Ezama et al. study, the results showed a notable distinct connection between the anterior segment of hippocampus (CA1, CA3, and DG sub-fields) and the anterior hippocampal network, a responsible network for scene processing and sensory information discrimination [18]. The current findings also demonstrate a clear positive functional connectivity between certain hippocampal sub-regions and the frontoparietal network. Specifically, the results indicate that only the EC sub-region had a positive functional connection with the frontoparietal network. This is an interesting observation since both the frontoparietal network and the EC sub-field of the hippocampus are implicated in memory retrieval, which is a crucial function of the hippocampus [47–52].

Another interesting finding of this study is that some of the functional connections between the hippocampal sub-regions and the remaining networks were heterogeneous and not consistently related to all sub-regions. This confirms the hippocampus's complex sub-structures with different cytoarchitectonic properties [18,53]. Additionally, the results demonstrate negative functional connectivity with some functional brain networks, particularly the salience network, which is involved in various complex functions such as awareness, saliency, social, and cognitive tasks [54,55]. The observation of negative functional connectivity or anticorrelated signals in rsfMRI is a topic of ongoing debate and research in the field of functional brain imaging. Anticorrelations represent negative correlations between the time courses of two brain regions or networks, suggesting that the activity in one region is suppressed or decreased when the activity in the other region increases [56,57]. While the interpretation of these anticorrelated signals is challenging, several potential explanations have been proposed including neural origin, artifacts and mixed neural and non-neural contributions [56–58]. While these findings are interesting and need further comprehensive analysis, it is beyond the aim of this study to investigate mainly due to the complex characterization and understanding of the anticorrelation signals in rsfMRI.

4.2. Similarities and differences among the sub-regions of the hippocampus as a factor of gender

The second part of the aim of this study is to investigate how gender differences affect the functional connectivity between hippocampal sub-regions and functional brain networks. Previous neuroimaging studies have reported sex differences in the volume of hippocampal sub-regions, suggesting that these differences may reflect differences in functional connectivity with cerebral and cerebellar networks between males and females [59,60].

While several studies have examined the influence of gender variations on the default mode network, the findings have been inconsistent [7,61,62]. Weissman-Fogel et al. investigated gender differences in the functional connectivity of the default mode network using a sample of 49 healthy participants and reported no sex differences in the functional connectivity of the default mode network. In contrast, Bluhm et al. found that females had higher functional connectivity in the medial prefrontal cortex and posterior cingulate cortex of the default mode network, whereas males did not demonstrate such connectivity. Additionally, Biswal et al. found that females had more functional connections in the medial prefrontal cortex of the default mode network. Furthermore, our findings revealed that sub-regions in males exhibited higher functional connectivity with the medial prefrontal cortex, while sub-regions in females demonstrated higher functional connectivity with the posterior cingulate cortex. In this study, the main sub-regions that displayed gender-based differences in functional connectivity with the default mode network were the left CA2 (males only), right CA2 (females only), left EC (males only), and right CA3 (females only). These differences may be attributed to the differential effects of gonadal steroids [63,64]. The observed gender differences in the functional connectivity of specific hippocampal sub-regions with the default mode network could be influenced by the modulatory effects of gonadal steroids. For example, estrogens have been shown to impact hippocampal structure and function, with a particular effect on synaptic plasticity and neurogenesis [65,66]. Testosterone, on the other hand, has been reported to influence hippocampal volume and spatial memory performance [67]. These hormonal effects on hippocampal sub-regions could lead to the observed gender differences in functional connectivity with the default mode network. It is important to note that while gonadal steroids might contribute to the observed gender differences, other factors, such as genetic, epigenetic, and environmental influences, could also play a role in shaping the functional connectivity of hippocampal sub-regions with the default mode network. Further studies investigating the specific mechanisms underlying these gender differences, including the role of gonadal steroids, would help elucidate the exact nature of these differences and their implications for brain function and cognitive processes.

It is noteworthy that these differences appear to be dominant on one side of the two hemispheres, particularly the right hemisphere in male subjects and the left hemisphere in female subjects. Our findings indicate strong positive functional connections between all of the visual network regions and the hippocampal sub-fields CA1, CA2, CA3, as well as the hippocampal-amygdaloid transition area (HATA). This could suggest that these specific hippocampal sub-regions play a crucial role in the processing and integration of visual information, which is supported by previous studies highlighting the involvement of the hippocampus in various visual tasks, such as scene processing and spatial navigation [68,69]. Moreover, our study demonstrated that these functional connections between the hippocampal sub-fields and visual networks were stronger in female subjects compared to male subjects. This observation aligns with

recent research that has identified gender differences in the functional connectivity of various brain regions, including the hippocampus, during tasks involving visual and emotional processing [70]. The stronger functional connections in female subjects may contribute to the differences in cognitive and behavioral outcomes between males and females in tasks that rely on visual processing and hippocampal function [23,71]. The identification of gender differences in the functional connectivity of hippocampal sub-regions with visual networks could have significant implications for understanding the neural basis of cognitive and behavioral variations between males and females. It also highlights the importance of considering gender as a factor in future research examining the functional connectivity and neural mechanisms underlying various cognitive processes. Our study provides valuable insights into the distinct functional roles of different hippocampal sub-fields in visual processing and reveals potential gender differences in the functional connectivity between hippocampal sub-regions and visual networks. These findings contribute to a deeper understanding of the complex interplay between hippocampal function and visual processing, paving the way for future investigations in this area.

The current data reveal that the EC sub-region of females is the only sub-region that positively connects with the frontoparietal network. In contrast, male sub-regions show no functional connectivity with all regions defining this network. The frontoparietal network is believed to play a role in a wide range of tasks by initiating and regulating cognitive control abilities [72]. It has been found to participate in working memory and direct attention [73–75]. Additionally, a previous study found that the hippocampus in females is more activated than in males during working memory [76]. These findings could explain why the EC sub-region of females' hippocampus shows a positive functional connection with the frontoparietal network. The findings also show that female sub-regions have more functional connectivity to the cerebellar network, which is recently recognized to be implicated not only in motor-associated controlling functions but also in higher-order functions such as working memory, sensory, and cognitive behaviors [77–80]. These results suggest that female sub-regions may have a higher role in working memory. This is in line with a study that investigated gender differences in working memory [76]. One further observation worth mentioning is that male sub-regions show higher functional connectivity with the sensorimotor network, which includes areas associated with speech function [81]. This confirms recent work indicating that the hippocampus plays a role in speech production [82].

4.3. Strength, limitations, and future directions

In this study, we have significantly advanced our understanding of the functional connectivity patterns of hippocampal sub-regions and their association with gender differences. One of the key strengths of our work is the relatively large sample size of 200 healthy subjects, which ensures a higher statistical power and generalizability of our findings. Additionally, we have utilized advanced neuroimaging techniques to investigate specific cytoarchitecturally distinct sub-regions of the hippocampus, rather than treating it as a single homogenous region. This approach provides a more detailed and nuanced understanding of the diverse functional roles played by different hippocampal sub-regions in various brain networks. Moreover, our study is among the first to explore the potential impact of gender differences on the functional connectivity of these hippocampal sub-regions. By examining the connectivity patterns of the sub-regions with eight major functional networks, we have identified clear similarities and differences between males and females, particularly in the anterior portion of the hippocampus. This novel focus on gender differences contributes to a growing body of research in neuroscience and has the potential to inform future studies on the neural basis of cognitive and behavioral variations between males and females. These methodological and analytical advances provide a more comprehensive understanding of the complex functional organization of the hippocampus and its implications for cognition and behavior.

The present study examined the functional connectivity patterns of seven sub-regions of the hippocampus in healthy individuals, as well as gender differences in these patterns. Although our results demonstrated significant differences in functional connectivity between these sub-regions and various functional brain networks, several limitations of our study should be considered. First, we only used resting-state fMRI data and did not examine functional connectivity during specific tasks. This may have limited our comprehension of the functional roles of these hippocampal sub-regions during different cognitive processes. Furthermore, one of the possible limitations of our study is the use of an *a priori* defined atlas for defining hippocampal sub-regions, rather than the more advanced and widely used FreeSurfer tool. While our method has been previously validated and used in the literature [31–33,83–85], it may not be as accurate as FreeSurfer. However, given the specific aims and scope of our study, we believe that our approach is suitable and appropriate for our research questions.

Despite these limitations, our study provides valuable insights into the functional connectivity patterns of the hippocampal sub-regions and gender differences in these patterns. Future studies could address these limitations by examining functional connectivity during task performance, and considering other sub-division methods of the hippocampus, such as functional connectivity-based parcellation or morphometric segmentation. Furthermore, future research could investigate the role of the hippocampal sub-regions in specific cognitive processes, such as episodic memory or spatial navigation, and how these processes may differ between males and females. Overall, the findings of our study have important implications for understanding the functional heterogeneity of the hippocampus and its role in gender-specific cognitive processes. Moreover, future studies could use more advanced tools, such as FreeSurfer, to segment hippocampal sub-fields and compare the results with our findings. Additionally, it would be beneficial to investigate the effects of different segmentation methods on the functional connectivity of hippocampal sub-regions. Further, it would be interesting to examine the relationship between hippocampal sub-fields connectivity and cognitive or clinical outcomes, such as memory performance or psychiatric disorders.

5. Conclusion

This study demonstrates the similarities and differences in functional connectivity among the seven sub-regions of the hippocampus

with major functional brain networks. It highlights the significant role of each hippocampus sub-region in visual and default mode functions. Moreover, it underscores the diverse implications of hippocampal sub-regions in other brain functions, suggesting that the hippocampus is not only a complex heterogeneous structure but also exhibits distinct functional changes within its sub-regions. Future studies should always consider investigating and accounting for these sub-fields of the hippocampus. Additionally, this study reveals similarities and differences between genders in these sub-regions based on functional connectivity to major brain functional networks. Specifically, the CA sub-fields and the EC exhibit distinct gender differences to the default mode and cerebellar network, indicating that gender differences should be considered when examining the physiological functional changes of the hippocampus.

Author contribution statement

Adnan Alahmadi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Nada O. Alotaibi; Norah Y. Hakami; Raghad S. Almutairi: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Afnan M. F. Darwesh; Rawan Abdeen; Osamah M. Abdulaal; Walaa Alsharif; Salahaden R Sultan: Contributed reagents, materials, analysis tools or data.

Jamaan Alghamdi; Ibrahim H. Kanbayti: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data associated with this study has been deposited at https://www.nitrc.org/projects/wakayama_psyrs.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research work was funded by Institutional Fund Projects under grant no (IFPRC-004-142-2020). Therefore, authors gratefully acknowledge technical and financial support from the Ministry of Education and King Abdulaziz University, Jeddah, Saudi Arabia.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2023.e20389>.

References

- [1] P.A. Schwartzkroin, Role of the hippocampus in epilepsy, *Hippocampus* 4 (3) (1994) 239–242.
- [2] K.S. Anand, V. Dhikav, Hippocampus in health and disease: an overview, *Ann. Indian Acad. Neurol.* 15 (4) (2012) 239.
- [3] S. Nakahara, et al., Hippocampal pathophysiology: commonality shared by temporal lobe epilepsy and psychiatric disorders, *Neurosci J* 2018 (2018), 4852359.
- [4] A. Disouky, O. Lazarov, Adult hippocampal neurogenesis in Alzheimer's disease, *Prog Mol Biol Transl Sci* 177 (2021) 137–156.
- [5] N.V. Malykhin, N.J. Coupland, Hippocampal neuroplasticity in major depressive disorder, *Neuroscience* 309 (2015) 200–213.
- [6] B. Biswal, et al., Functional connectivity in the motor cortex of resting human brain using echo-planar MRI, *Magn. Reson. Med.* 34 (4) (1995) 537–541.
- [7] B.B. Biswal, et al., Toward discovery science of human brain function, *Proc. Natl. Acad. Sci. USA* 107 (10) (2010) 4734–4739.
- [8] K.J. Friston, Modalities, modes, and models in functional neuroimaging, *Science* 326 (5951) (2009) 399–403.
- [9] W. Yin, L. Li, F.-X. Wu, Deep learning for brain disorder diagnosis based on fMRI images, *Neurocomputing* 469 (2022) 332–345.
- [10] J.P. Niskanen, et al., Monitoring functional impairment and recovery after traumatic brain injury in rats by FMRI, *J. Neurotrauma* 30 (7) (2013) 546–556.
- [11] S.M. Smith, Overview of fMRI analysis, *The British Journal of Radiology* 77 (suppl.2) (2004) S167–S175.
- [12] R.A. Poldrack, The role of fMRI in Cognitive Neuroscience: where do we stand? *Curr. Opin. Neurobiol.* 18 (2) (2008) 223–227.
- [13] A.F. Wolters, et al., Resting-state fMRI in Parkinson's disease patients with cognitive impairment: a meta-analysis, *Parkinsonism Relat Disord* 62 (2019) 16–27.
- [14] D. Pinter, et al., Reproducibility of resting state connectivity in patients with stable multiple sclerosis, *PLoS One* 11 (3) (2016), e0152158.
- [15] F. Gonçalves Ó, M.C. Batistuzzo, J.R. Sato, Real-time functional magnetic resonance imaging in obsessive-compulsive disorder, *Neuropsychiatr Dis Treat* 13 (2017) 1825–1834.
- [16] J.P. Kuhtz-Buschbeck, et al., Brain activity is similar during precision and power gripping with light force: an fMRI study, *Neuroimage* 40 (4) (2008) 1469–1481.
- [17] E.M. Blessing, et al., A data-driven approach to mapping cortical and subcortical intrinsic functional connectivity along the longitudinal hippocampal axis, *Hum. Brain Mapp.* 37 (2) (2016) 462–476.
- [18] L. Ezama, et al., Functional connectivity of the hippocampus and its subfields in resting-state networks, *Eur. J. Neurosci.* 53 (10) (2021) 3378–3393.
- [19] W.-T. Chang, et al., Brainwide functional networks associated with anatomically-and functionally-defined hippocampal subfields using ultrahigh-resolution fMRI, *Sci. Rep.* 11 (1) (2021) 1–13.
- [20] R. Vos de Wael, et al., Anatomical and microstructural determinants of hippocampal subfield functional connectome embedding, *Proc. Natl. Acad. Sci. USA* 115 (40) (2018) 10154–10159.
- [21] R. de Flores, et al., Intrinsic connectivity of hippocampal subfields in normal elderly and mild cognitive impairment patients, *Hum. Brain Mapp.* 38 (10) (2017) 4922–4932.

- [22] M. Nini, et al., Gender differences in dynamic functional connectivity based on resting-state fMRI, *Annu Int Conf IEEE Eng Med Biol Soc* 2017 (2017) 2940–2943.
- [23] S. Yagi, L.A.M. Galea, Sex differences in hippocampal cognition and neurogenesis, *Neuropsychopharmacology* 44 (1) (2019) 200–213.
- [24] R.C. Gur, et al., Sex differences in brain gray and white matter in healthy young adults: correlations with cognitive performance, *J. Neurosci.* 19 (10) (1999) 4065–4072.
- [25] S.J. Ritchie, et al., Sex differences in the adult human brain: evidence from 5216 UK biobank participants, *Cerebr. Cortex* 28 (8) (2018) 2959–2975.
- [26] L. van Eijk, et al., Region-specific sex differences in the hippocampus, *Neuroimage* 215 (2020), 116781.
- [27] L. Cahill, et al., Sex-related difference in amygdala activity during emotionally influenced memory storage, *Neurobiol. Learn. Mem.* 75 (1) (2001) 1–9.
- [28] G. Gong, et al., Age- and gender-related differences in the cortical anatomical network, *J. Neurosci.* 29 (50) (2009), 15684.
- [29] S. Zhang, R.L. Chiang-shan, Functional connectivity mapping of the human precuneus by resting state fMRI, *Neuroimage* 59 (4) (2012) 3548–3562.
- [30] L. Cahill, et al., The influence of sex versus sex-related traits on long-term memory for gist and detail from an emotional story, *Conscious. Cognit.* 13 (2) (2004) 391–400.
- [31] K. Amunts, et al., Cytoarchitectonic mapping of the human amygdala, hippocampal region and entorhinal cortex: intersubject variability and probability maps, *Anat. Embryol.* 210 (5) (2005) 343–352.
- [32] K. Zilles, K. Amunts, Centenary of Brodmann's map—conception and fate, *Nat. Rev. Neurosci.* 11 (2) (2010) 139–145.
- [33] S.B. Eickhoff, et al., A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data, *Neuroimage* 25 (4) (2005) 1325–1335.
- [34] T. Donishi, M. Terada, Y. Kaneoke, Effects of gender, digit ratio, and menstrual cycle on intrinsic brain functional connectivity: a whole-brain, voxel-wise exploratory study using simultaneous local and global functional connectivity mapping, *Brain and Behavior* 8 (1) (2018), e00890.
- [35] S. Whitfield-Gabrieli, A. Nieto-Castanon, Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks, *Brain Connect.* 2 (3) (2012) 125–141.
- [36] N.B. Turk-Browne, The hippocampus as a visual area organized by space and time: a spatiotemporal similarity hypothesis, *Vision Res* 165 (2019) 123–130.
- [37] Z.-X. Liu, et al., Visual sampling predicts hippocampal activity, *J. Neurosci.* 37 (3) (2017) 599.
- [38] D.E. Hannula, C. Ranganath, The eyes have it: hippocampal activity predicts expression of memory in eye movements, *Neuron* 63 (5) (2009) 592–599.
- [39] D.C. Haggerty, D. Ji, Activities of visual cortical and hippocampal neurons co-fluctuate in freely moving rats during spatial behavior, *Elife* 4 (2015), e08902.
- [40] W. Huijbers, et al., The hippocampus is coupled with the default network during memory retrieval but not during memory encoding, *PLoS One* 6 (4) (2011), e17463.
- [41] G.A. James, et al., Diminished default mode network recruitment of the hippocampus and parahippocampus in temporal lobe epilepsy, *J. Neurosurg.* 119 (2) (2013) 288–300.
- [42] M.B. Moser, E.I. Moser, Functional differentiation in the hippocampus, *Hippocampus* 8 (6) (1998) 608–619.
- [43] I. Kahn, et al., Distinct cortical anatomy linked to subregions of the medial temporal lobe revealed by intrinsic functional connectivity, *J. Neurophysiol.* 100 (1) (2008) 129–139.
- [44] S. Qin, et al., Large-scale intrinsic functional network organization along the long axis of the human medial temporal lobe, *Brain Struct. Funct.* 221 (6) (2016) 3237–3258.
- [45] A. Lee, L.-K. Yeung, M. Barense, The hippocampus and visual perception, *Front. Hum. Neurosci.* 6 (2012).
- [46] D.D. Burman, Hippocampal connectivity with sensorimotor cortex during volitional finger movements: laterality and relationship to motor learning, *PLoS One* 14 (9) (2019), e0222064.
- [47] S. Konishi, et al., Neural correlates of episodic retrieval success, *Neuroimage* 12 (3) (2000) 276–286.
- [48] K.B. McDermott, et al., Retrieval success is accompanied by enhanced activation in anterior prefrontal cortex during recognition memory: an event-related fMRI study, *J. Cogn. Neurosci* 12 (6) (2000) 965–976.
- [49] D.I. Donaldson, S.E. Petersen, R.L. Buckner, Dissociating memory retrieval processes using fMRI: evidence that priming does not support recognition memory, *Neuron* 31 (6) (2001) 1047–1059.
- [50] V.H. Brun, et al., Place cells and place recognition maintained by direct entorhinal-hippocampal circuitry, *Science* 296 (5576) (2002) 2243–2246.
- [51] O.Y. Chao, et al., The medial prefrontal cortex-lateral entorhinal cortex circuit is essential for episodic-like memory and associative object-recognition, *Hippocampus* 26 (5) (2016) 633–645.
- [52] S.E. Qasim, et al., Memory retrieval modulates spatial tuning of single neurons in the human entorhinal cortex, *Nat. Neurosci.* 22 (12) (2019) 2078–2086.
- [53] J.P. Aggleton, Multiple anatomical systems embedded within the primate medial temporal lobe: implications for hippocampal function, *Neurosci. Biobehav. Rev.* 36 (7) (2012) 1579–1596.
- [54] L.Q. Uddin, *Salience Network of the Human Brain*, Academic press, 2016.
- [55] W.W. Seeley, The salience network: a neural system for perceiving and responding to homeostatic demands, *J. Neurosci.* 39 (50) (2019) 9878–9882.
- [56] M.D. Fox, et al., The human brain is intrinsically organized into dynamic, anticorrelated functional networks, *Proc. Natl. Acad. Sci. USA* 102 (27) (2005) 9673–9678.
- [57] K. Murphy, M.D. Fox, Towards a consensus regarding global signal regression for resting state functional connectivity MRI, *Neuroimage* 154 (2017) 169–173.
- [58] A. Weissenbacher, et al., Correlations and anticorrelations in resting-state functional connectivity MRI: a quantitative comparison of preprocessing strategies, *Neuroimage* 47 (4) (2009) 1408–1416.
- [59] J. Persson, et al., Sex differences in volume and structural covariance of the anterior and posterior hippocampus, *Neuroimage* 99 (2014) 215–225.
- [60] J. Sacher, et al., Sexual dimorphism in the human brain: evidence from neuroimaging, *Magn. Reson. Imaging* 31 (3) (2013) 366–375.
- [61] I. Weissman-Fogel, et al., Cognitive and default-mode resting state networks: do male and female brains “rest” differently? *Hum. Brain Mapp.* 31 (11) (2010) 1713–1726.
- [62] R.L. Bluhm, et al., Default mode network connectivity: effects of age, sex, and analytic approach, *Neuroreport* 19 (8) (2008) 887–891.
- [63] H.E. Scharfman, N.J. MacLusky, Sex differences in hippocampal area CA3 pyramidal cells, *J. Neurosci. Res.* 95 (1–2) (2017) 563–575.
- [64] P. Duarte-Guterman, et al., Hippocampal learning, memory, and neurogenesis: effects of sex and estrogens across the lifespan in adults, *Horm. Behav.* 74 (2015) 37–52.
- [65] C.S. Woolley, B.S. McEwen, Estradiol regulates hippocampal dendritic spine density via an N-methyl-D-aspartate receptor-dependent mechanism, *J. Neurosci.* 14 (12) (1994) 7680–7687.
- [66] L.A. Galea, Gonadal hormone modulation of neurogenesis in the dentate gyrus of adult male and female rodents, *Brain Res. Rev.* 57 (2) (2008) 332–341.
- [67] M.D. Spritzer, L.A. Galea, Testosterone and dihydrotestosterone, but not estradiol, enhance survival of new hippocampal neurons in adult male rats, *Developmental neurobiology* 67 (10) (2007) 1321–1333.
- [68] E.A. Maguire, S.L. Mullally, The hippocampus: a manifesto for change, *J. Exp. Psychol. Gen.* 142 (4) (2013) 1180.
- [69] E.M. Aminoff, K. Kveraga, M. Bar, The role of the parahippocampal cortex in cognition, *Trends Cognit. Sci.* 17 (8) (2013) 379–390.
- [70] J. Zhang, et al., Gender differences in global functional connectivity during facial emotion processing: a visual MMN study, *Front. Behav. Neurosci.* 12 (2018) 220.
- [71] A. Grabowska, Sex on the brain: are gender-dependent structural and functional differences associated with behavior? *J. Neurosci. Res.* 95 (1–2) (2017) 200–212.
- [72] N.U. Dosenbach, et al., A dual-networks architecture of top-down control, *Trends Cognit. Sci.* 12 (3) (2008) 99–105.
- [73] T.D. Wager, E.E. Smith, Neuroimaging studies of working memory: a meta-analysis, *Cogn Affect Behav Neurosci* 3 (4) (2003) 255–274.
- [74] A.M. Owen, et al., N-back working memory paradigm: a meta-analysis of normative functional neuroimaging studies, *Hum. Brain Mapp.* 25 (1) (2005) 46–59.
- [75] C. Rottschy, et al., Modelling neural correlates of working memory: a coordinate-based meta-analysis, *Neuroimage* 60 (1) (2012) 830–846.
- [76] A.C. Hill, A.R. Laird, J.L. Robinson, Gender differences in working memory networks: a BrainMap meta-analysis, *Biol. Psychol.* 102 (2014) 18–29.
- [77] S.P. Tomlinson, et al., Cerebellar contributions to verbal working memory, *Cerebellum* 13 (3) (2014) 354–361.

- [78] B. Devereett, et al., Cerebellar disruption impairs working memory during evidence accumulation, *Nat. Commun.* 10 (1) (2019) 3128.
- [79] J.H. Balsters, et al., Evolution of the cerebellar cortex: the selective expansion of prefrontal-projecting cerebellar lobules, *Neuroimage* 49 (3) (2010) 2045–2052.
- [80] C. Habas, Functional imaging of the deep cerebellar nuclei: a review, *Cerebellum* 9 (1) (2010) 22–28.
- [81] R. Behroozmand, et al., Sensory-motor networks involved in speech production and motor control: an fMRI study, *Neuroimage* 109 (2015) 418–428.
- [82] V. van de Ven, L. Waldorp, I. Christoffels, Hippocampus plays a role in speech feedback processing, *Neuroimage* 223 (2020), 117319.
- [83] T. Wolbers, et al., Differential recruitment of the hippocampus, medial prefrontal cortex, and the human motion complex during path integration in humans, *J. Neurosci.* 27 (35) (2007) 9408–9416.
- [84] N. Palomero-Gallagher, et al., Multimodal mapping and analysis of the cyto-and receptorarchitecture of the human hippocampus, *Brain Struct. Funct.* 225 (2020) 881–907.
- [85] K.A. Koenig, et al., The role of the thalamus and hippocampus in episodic memory performance in patients with multiple sclerosis, *Multiple Sclerosis Journal* 25 (4) (2019) 574–584.